Sporadic Development of Ustilago striiformis in Axillary Buds from Stolon Nodes of Perennially Infected Agrostis palustris

Clinton F. Hodges

Assistant Professor of Horticulture and Agronomy, Departments of Horticulture and Agronomy, Iowa State University, Ames 50010.

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ABSTRACT

Axillary buds from stripe-smutted and control stolons of *Agrostis palustris* were observed for survival and growth on 12 organ culture media. Three media consisting of inorganic salts, dextrose and/or levulose, and gibberellic acid supported satisfactory bud growth. Growth of axillary buds from stripe-smutted stolons established that infection of axillary buds by *Ustilago striiformis* var. *agrostidis* was sporadic. Of the stolons produced on all media by surviving axillary buds from stripe-smutted plants, 56% were healthy. It was hypothesized that infection of axillary buds by *U. striiformis* var. *agrostidis* was subject to loss of apical dominance in stolons. Phytopathology 61:940-942.

Additional key words: organ culture, etiology.

Ustilago striiformis (West.) Niessl is well known as a perennial pathogen of numerous species of Gramineae (1, 2, 4, 5, 6). In *Agrostis palustris* Hud., mycelium colonizes stolon nodes and subsequently grows into stolons produced from axillary buds on nodes (2, 3). Histopathological studies of stripe-smutted *A. palustris* have failed to demonstrate the presence of *U. striiformis* var. *agrostidis* in axillary buds attached to mycelium-colonized nodes (2). This is peculiar, as the pathogen infects all other aboveground, vegetative structures of *A. palustris* (2). It is possible that mycelium does not enter meristems until growth is initiated; however, it is also possible that conventional histological techniques are inadequate for detecting mycelium in meristems. The purpose of this research was to further examine the relationship between mycelium-colonized nodes and their adjoining buds by means of direct observation of plants produced by axillary buds removed from stripe-smutted stolons and grown in organ culture.

MATERIALS AND METHODS.—Stripe-smutted *A. palustris* (Old Orchard) was used for all studies. All stolons were greenhouse-grown (13-36 C), and axillary buds were removed from the six youngest visible nodes by means of a microdissecting knife. Buds ranged from 1.2 to 2.9 mm in length and were collected only from the axils of stripe-smutted leaves. Control buds were secured in the same manner. All buds were surface-sterilized 8-10 min each in 0.5% cetyl trimethyl ammonium bromide and 10% Clorox (5.25% sodium hypochlorite) solutions, then washed in sterile, double-distilled water and placed on organ culture media.

Bud growth and development was observed on 12 organ culture media. All media contained White's salts solution (7), 2.0 mg/liter gibberellic acid (K salt), and 50 g/liter Difco-Bacto agar. Media were divided into three lots on the basis of sugar constituents. Each lot contained 10 g/liter of either dextrose, levulose, or a combination of dextrose (5 g/liter) and levulose (5 g/liter). Each of these lots was further subdivided into four sublots; each of which contained either casein hydrolysate (4 g/liter), yeast hydrolysate (4 g/liter), or corn hydrolysate (4 ml/liter). The fourth sublot contained sugar or sugar combinations only. Twenty ml of each medium were placed in each of twenty-five 25- × 150-mm culture tubes, then autoclaved 30 min, and allowed to solidify on a slant. Gibberellic acid was added to the media prior to solidification by means of a syringe equipped with a Millipore filter. One bud was placed in each of 25 tubes on each medium and grown for 50 days. All cultures were incubated at 24.0 C (± 1.0 C) under a 14-hr day (400 ft-c). Cultures were evaluated for bud survival and the presence of stripe-smut sori in stolons produced.

RESULTS.—*Bud survival on culture media.*—Total survival of buds from control and stripe-smutted stolons on all culture media evaluated was low. Of 300 buds each, from stripe-smutted and control stolons, only 36% and 34% survived, respectively. Although total bud survival on all media was low, media containing dextrose only, levulose only, and a combination of dextrose and levulose only, proved satisfactory (Table 1). Survival of buds from stripe-smutted and control stolons, on dextrose only, was 84% and 76%, respectively; on levulose only, 72% and 84%, respectively, for each medium. Bud survival, on the combination of dextrose and levulose only, was 64 and 72% from stripe-smutted and control stolons, respectively. Hydrolysate media were generally unsatisfactory; survival of buds from control and stripe-smutted stolons ranged from 0 to 12 and 0 to 14, respectively. Among hydrolysate media, bud survival was maximum on levulose-corn hydrolysate medium; survival of buds from stripe-smutted and control stolons was 56% and 48%, respectively. Most cultures that did not survive were lost directly to bud necrosis, with culture contamination accounting for 0-4 buds/medium.

Development of stripe-smutted stolons from buds.—Buds from stripe-smutted and control plants produced a total of 132 and 113 stolons, respectively, on all media. Buds from stripe-smutted plants produced individual cultures of healthy and smutted stolons (Fig. 1). Of the stolons produced on all media from buds of stripe-smutted stolons, 56% were healthy. Production of healthy stolons from buds of stripe-smutted stolons

TABLE 1. Survival and development of axillary buds from nodes of healthy and stripe-smutted stolons of *Agrostis palustris* on organ culture media containing sugar and sugar combinations only

	Surviving buds from control and stripe-smutted plants and their production of stolons[a]								
	Control				Stripe-smutted				
Culture media[b]	Necrotic buds	Surviving buds	Healthy stolons/ surviving bud	Avg no. stolons/ surviving bud	Necrotic buds	Surviving buds	Healthy stolons/ surviving bud	Stripe-smutted stolons/ surviving bud	Avg no. stolons/ surviving bud
Dextrose only	6	19 (76%)	25	1.3	4	21 (84%)	14 (52%)	13 (48%)	1.3
Levulose only	4	21 (84%)	25	1.2	7	18 (72%)	8 (32%)	17 (68%)	1.4
Dextrose-levulose only	7	18 (72%)	19	1.1	9	16 (64%)	13 (52%)	12 (48%)	1.6

[a] 25 Buds were placed on each medium; cultures lost to contamination are included with necrotic buds and ranged from 0-4/medium. All media contained White's (7) salt solution, 2.0 mg/liter gibberellic acid (K salt), and 50 g/liter Difco-Bacto agar.

[b] Comparable lots containing either casein hydrolysate (4 g/liter), yeast hydrolysate (4 g/liter), or corn hydrolysate (4 ml/liter) with each sugar or sugar combination were unsatisfactory.

was somewhat lower on media containing dextrose only, levulose only, and dextrose-levulose only (Table 1). Maximum stolon numbers also occurred on media containing dextrose only, levulose only, and dextrose-levulose only, and was indicative of greater bud survival. The average number of healthy and diseased stolons produced from buds of control and stripe-smutted plants was also greatest on these media; the average number of stolons ranged from 1.1 to 1.6 (Table 1). Buds on hydrolysate media generally produced one stolon/surviving bud; the only exceptions occurring on levulose-corn hydrolysate and dextrose-levulose-casein hydrolysate media on which buds from stripe-smutted plants produced 1.1 stolons/bud.

DISCUSSION.—Meristems of *A. palustris* can be grown on relatively simple organ culture media. Survival of meristems on media containing only sugars was satisfactory (Table 1); media containing hydrolysates were unacceptable. Media evaluation in this study was conducted for the sole purpose of finding satisfactory substrate for bud growth. Therefore, no attempt is being made to provide a physiological explanation of bud survival on various media. There is, however, little doubt that media containing dextrose only, or levulose only, were superior to all other media. Survival and production of both stripe-smutted and healthy stolons from axillary buds of stripe-smutted stolons on the various organ culture media are indicative of potential host-pathogen interactions which warrant further interpretation.

Two facts have been established on which future studies might be based: (i) The presence of *U. striiformis* in axillary buds of perennially infected stolons of *A. palustris* is sporadic; and (ii) organ culture can be used to study the etiological interactions of *U. striiformis* and the meristems of *A. palustris*. Since all other aboveground vegetative parts of *A. palustris* are infected by *U. striiformis* (2), it is difficult to explain why only 44% of the stolons produced from buds of

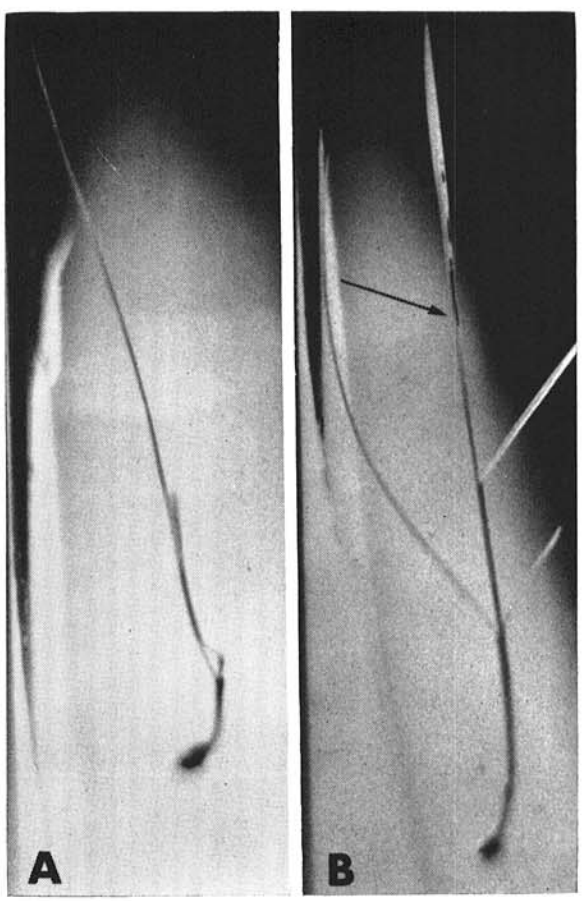

Fig. 1. Plants grown from axillary buds of stripe-smutted stolons of *Agrostis palustris*. **A)** Healthy plant produced from a bud not infected by *Ustilago striiformis*. **B)** Stripe-smutted plant showing sorus (arrow) produced from an infected bud.

stripe-smutted stolons were diseased. It is probable that these results are directly associated with interacting growth characteristics of the pathogen and host. *Ustilago striiformis* colonizes and proliferates in node tissues of *A. palustris* and *Poa pratensis* (2, 4); this characteristic, combined with the inactive state of axillary buds (due to apical dominance), provides one possible explanation. Assuming that *U. striiformis* is incapable of entering inactive buds (assumption is based on the 56% healthy stolons produced from buds of stripe-smutted stolons), subsequent infection of axillary buds may be directly dependent upon loss of apical dominance. It is not possible to determine when active bud growth is about to begin, but because of the progressive loss of apical dominance from the basal to terminal ends of stolons, infection of axillary buds farthest from the terminal bud would be expected first and before visible bud growth.

The hypothesis is plausible, and could account for the 44% stripe-smutted and 56% healthy stolons, respectively, produced from buds of diseased plants. The possible interaction between *U. striiformis* and apical dominance of the host may also be subject to temperature. Studies of temperature influence on growth of *U. striiformis* in *A. palustris* showed that day-night temperatures of 37.8 and 32.2 C will inhibit growth of the pathogen in stolons (3). The experiments reported herein were conducted over 11 months with greenhouse-grown stolons at temperatures ranging from 13-36 C; it is probable that temperature inhibition of *U. striiformis* was involved.

By eliminating the temperature variable, the presence of *U. striiformis* in axillary buds relative to apical dominance should be demonstrable in organ culture. If *U. striiformis* infects only active buds, the terminal or apical buds of diseased stolons should always be infected. If the interaction between *U. striiformis* and apical dominance exists as hypothesized, apical buds should produce only stripe-smutted stolons, and axillary buds should produce increasing numbers of diseased stolons in descending order from the youngest to oldest buds.

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